8EHQ-0992-13141

(1)

FORM LG-4878

DU PONT

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E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

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Environmental Protection Agency
401 M Street., S.W.
Washington, D.C. 20460
Attn: Section 8(e) Coordinator (CAP Agreement)

Dear Coordinator:

8ECAP-0025

On behalf of the Regulatee and pursuant to Unit II B.1.b. and Unit II C of the 6/28/91CAP Agreement, E.I. Du Pont de Nemours and Co. hereby submits (*in triplicate*) the attached studies. Submission of this information is voluntary and is occasioned by unilateral changes in EPA's standard as to what EPA now considers as reportable information. Regulatee's submission of information is made solely in response to the new EPA §8(e) reporting standards and is not an admission: (1) of TSCA violation or liability; (2) that Regulatee's activities with the study compounds reasonably support a conclusion of substantial health or environmental risk or (3) that the studies themselves reasonably support a conclusion of substantial health or environmental risk.

The "Reporting Guide" creates new TSCA 8(e) reporting criteria which were not previously announced by EPA in its 1978 Statement of Interpretation and Enforcement Policy, 43 Fed Reg 11110 (March 16, 1978). The "Reporting Guide states criteria which expands upon and conflicts with the 1978 Statement of Interpretation. Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" raises significant due processes issues and clouds the appropriate reporting standard by which regulated persons can assure TSCA Section 8(e) compliance.

For Regulatee,

8ECAP

Mark H. Christman
Counsel
Legal D-7158
1007 Market Street
Wilmington, DE 19898
(302) 774-6443

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3/24/95

ATTACHMENT 1

Submission of information is made under the 6/28/91 CAP Agreement, Unit II. This submission is made voluntarily and is occasioned by recent changes in EPA's TSCA §8(e) reporting standard; such changes made, for the first time in 1991 and 1992 without prior notice and in violation of Regulatee's constitutional due process rights. Regulatee's submission of information under this changed standard is not a waiver of its due process rights; an admission of TSCA violation or liability, or an admission that Regulatee's activities with the study compounds reasonably support a conclusion of substantial risk to health or to the environment. Regulatee has historically relied in good faith upon the 1978 Statement of Interpretation and Enforcement Policy criteria for determining whether study information is reportable under TSCA §8(e), 43 Fed Reg 11110 (March 16, 1978). EPA has not, to date, amended this Statement of Interpretation.

After CAP registration, EPA provided the Regulatee the June 1, 1991 "TSCA Section 8(e) Reporting Guide". This "Guide" has been further amended by EPA, EPA letter, April 10, 1992. EPA has not indicated that the "Reporting Guide" or the April 1992 amendment supersedes the 1978 Statement of Interpretation. The "Reporting Guide" and April 1992 amendment substantively lowers the Statement of Interpretation 's TSCA §8(e) reporting standard[2]. This is particularly troublesome as the "Reporting Guide" states criteria, applied retroactively, which expands upon and conflicts with the Statement of Interpretation.[3] Absent amendment of the Statement of Interpretation, the informal issuance of the "Reporting Guide" and the April 1992 amendment clouds the appropriate standard by which regulated persons must assess information for purposes of TSCA §8(e).

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[2]In sharp contrast to the Agency's 1977 and 1978 actions to soliciting public comment on the proposed and final §8(e) Policy, EPA has unilaterally pronounced §8(e) substantive reporting criteria in the 1991 Section 8(e) Guide without public notice and comment, See 42 Fed Reg 45362 (9/9/77), "Notification of Substantial Risk under Section 8(e): Proposed Guidance".

[3]A comparison of the 1978 Statement of Interpretation and the 1992 "Reporting Guide" is a appended.

Throughout the CAP, EPA has mischaracterized the 1991 guidance as reflecting "longstanding" EPA policy concerning the standards by which toxicity information should be reviewed for purposes of §8(e) compliance. Regulatee recognizes that experience with the 1978 Statement of Interpretation may cause a review of its criteri. Regulatee supports and has no objection to the Agency's amending reporting criteria *provided that* such amendment is not applied to the regulated community in an unfair way. However, with the unilateral announcement of the CAP under the auspices of an OCM enforcement proceeding, EPA has wrought a terrific unfairness since much of the criteria EPA has espoused in the June 1991 Reporting Guide and in the Agency's April 2, 1992 amendment is new criteria which does not.exist in the 1978 Statement of Interpretation and Enforcement Policy.

The following examples of new criteria contained in the "Reporting Guide" that is not contained in the Statement of Interpretation follow:

- o even though EPA expressly disclaims each "status report" as being preliminary evaluations that should not be regarded as final EPA policy or intent[4], the "Reporting Guide" gives the "status reports" great weight as "sound and adequate basis" from which to determine mandatory reporting obligations. ("Guide" at page 20).
- o the "Reporting Guide" contains a matrix that establishes new numerical reporting "cutoff" concentrations for acute lethality information ("Guide" at p. 31). Neither this matrix nor the cutoff values therein are contained in the Statement of Interpretation. The regulated community was not made aware of these cutoff values prior to issuance of the "Reporting Guide" in June, 1991.
- othe "Reporting Guide" states new specific definitional criteria with which the Agency, for the first time, defines as 'distinguishable neurotoxicological effects'; such criteria/guidance not expressed in the 1978 Statement of Interpretation.[5];

- othe "Reporting Guide" provides new review/ reporting criteria for irritation and sensitization studies; such criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy.
- othe "Reporting Guide" publicizes certain EPA Q/A criteria issued to the Monsanto Co. in 1989 which are not in the Statement of Interpretation; have never been published in the Federal Register or distributed by the EPA to the Regulatee. Such Q/A establishes new reporting criteria not previously found in the 1978 Statement of Interpretation/Enforcement Policy .

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[4]The 'status reports' address the significance, if any, of particular information reported to the Agency, rather than stating EPA's interpretation of §8(e) reporting criteria. In the infrequent instances in which the status reports contain discussion of reportability, the analysis is invariably quite limited, without substantial supporting scientific or legal rationale.

[5] *See, e.g,* 10/2/91 letter from Du Pont to EPA regarding the definition of 'serious and prolonged effects' as this term may relate to transient anesthetic effects observed at lethal levels; 10/1/91 letter from the American Petroleum Institute to EPA regarding clarification of the Reporting Guide criteria.

In discharging its responsibilities, an administrative agency must give the regulated community fair and adequate warning to as what constitutes noncompliance for which penalties may be assessed.

> Among the myriad applications of the due process clause is the fundamental principle that statutes and regulations which purport to govern conduct must give an adequate warning of what they command or forbid.... Even a regulation which governs purely economic or commercial activities, if its violation can engender penalties, must be so framed as to provide a constitutionally adequate warning to those whose activities are governed.

Diebold, Inc. v. Marshall, 585 F.2d 1327, 1335-36 (D.C. Cir. 1978). *See also*, Rollins Environemntal Services (NJ) Inc. v. U.S. Environmental Protection Agency, 937 F. 2d 649 (D.C. Cir. 1991).

While neither the are rules, This principle has been applied to hold that agency 'clarification', such as the Statement of Interpretation, the "Reporting Guide" nor the April 1992 amendments will not applied retroactively.

> ...a federal court will not retroactively apply an unforeseeable interpretation of an administrative regulation to the detriment of a regulated party on the theory that the post hoc interpretation asserted by the Agency is generally consistent with the policies underlying the Agency's regulatory program, when the semantic meaning of the regulations, as previously drafted and construed by the appropriate agency, does not support the interpretation which that agency urges upon the court.

Standard Oil Co. v. Federal Energy Administration, 453 F. Supp. 203, 240 (N.D. Ohio 1978), aff'd sub nom. Standard Oil Co. v. Department of Energy, 596 F.2d 1029 (Em. App. 1978):

The 1978 Statement of Interpretation does not provide adequate notice of, and indeed conflicts with, the Agency's current position at §8(e) requires reporting of all 'positive' toxicological findings without regard to an assessment of their relevance to human health. In accordance with the statute, EPA's 1978 Statement of Interpretation requires the regulated community to use scientific judgment to evaluate the significance of toxicological findings and to determining whether they reasonably support a conclusion of a substantial risk. Part V of the Statement of Interpretation urges persons to consider "the fact or probability" of an effect's occurrence. Similarly, the 1978 Statement of Interpretation stresses that an animal study is reportable only when "it contains reliable evidence ascribing the effect to the chemical." 43 Fed Reg. at 11112. Moreover, EPA's Statement of Interpretation defines the substantiality of risk as a function of both the seriousness of the effect and the probability of its occurrence. 43 Fed Reg 11110 (1978). Earlier Agency interpretation also emphasized the "substantial" nature of a §8(e) determination. See 42 Fed Reg 45362, 45363

(1977). [Section 8(e) findings require "extraordinary exposure to a chemical substance...which critically imperil human health or the environment"].

The recently issued "Reporting Guide" and April 1992 Amendment guidance requires reporting beyond and inconsistent with that required by the Statement of Interpretation. Given the statute and the Statement of Interpretation's explicit focus on substantial human or environmental risk, whether a substance poses a "substantial risk" of injury requires the application of scientific judgment to the available data on a case-by-case basis.

If an overall weight-of-evidence analysis indicates that this classification is unwarranted, reporting should be unnecessary under §8(e) because the available data will not "reasonably support the conclusion" that the chemical presents a substantial risk of serious adverse consequences to human health.

Neither the legislative history of §8(e) nor the plain meaning of the statute support EPA's recent lowering of the reporting threshold that TSCA §8(e) was intended to be a sweeping information gathering mechanism. In introducing the new version of the toxic substances legislation, Representative Eckhart included for the record discussion of the specific changes from the version of H. R. 10318 reported by the Consumer Protection and Finance Subcommittee in December 1975. One of these changes was to modify the standard for reporting under §8(e). The standard in the House version was changed from "causes or contributes to an unreasonable risk" to "causes or significantly contributes to a substantial risk". This particular change was one of several made in TSCA §8 to avoid placing an undue burden on the regulated community. The final changes to focus the scope of Section 8(e) were made in the version reported by the Conference Committee.

The word "substantial" means "considerable in importance, value, degree, amount or extent". Therefore, as generally understood, a "substantial risk" is one which will affect a considerable number of people or portion of the environment, will cause serious injury and is based on reasonably sound scientific analysis or data. Support for the interpretation can be found in a similar provision in the Consumer Product Safety Act. Section 15 of the CPSA defines a "substantial product hazard" to be:

> "a product defect which because of the pattern of defect, the number of defective products distributed in commerce, the severity of the risk, or otherwise, creates a substantial risk of injury to the public."

Similarly, EPA has interpreted the word 'substantial' as a quantitative measurement. Thus, a 'substantial risk' is a risk that can be quantified, *See,* 56 Fed Reg 32292, 32297 (7/15/91). Finally, since information pertinent to the exposure of humans or the environment to chemical substances or mixtures may be obtained by EPA through Sections 8(a) and 8(d) regardless of the degree of potential risk, §8(e) has specialized function. Consequently, information subject to §8(e) reporting should be of a type which would lead a reasonable man to conclude that some type action was required immediately to prevent injury to health or the environment.

Attachment

Comparison:

Reporting triggers found in the 1978 "Statement of Interpretation/ Enforcement Policy",43 Fed Reg 11110 (3/16/78) and the June 1991 *Section 8(e) Guide.*

TEST TYPE	1978 POLICY CRITERIA EXIST?	New 1991 GUIDE CRITERIA EXIST?
ACUTE LETHALITY		
Oral	N}	Y}
Dermal	N}	Y}
Inhalation (Vapors)	}[6]	}[7]
aerosol	N}	Y}
dusts/ particles	N}	Y}
SKIN IRRITATION	N	Y[8]
SKIN SENSITIZATION (ANIMALS)	N	Y[9]
EYE IRRITATION	N	Y[10]
SUBCHRONIC (ORAL/DERMAL/INHALATION)	N	Y[11]
REPRODUCTION STUDY	N	Y[12]
DEVELOPMENTAL TOX	Y[13]	Y[14]

[6] 43 Fed Reg at 11114, comment 14:

"This policy statements directs the reporitng of specifiec effects when unknown to the Administrator. Many routine tests are based on a knowledge of toxicity associated with a chemicalL unknown effects occurring during such a range test may have to be reported if they are those of concern tot he Agency and if the information meets the criteria set forth in Parts V and VII."

[7] Guide at pp.22, 29-31.

[8] Guide at pp-34-36.

[9] Guide at pp-34-36.

[10] Guide at pp-34-36.

[11] Guide at pp-22; 36-37.

[12] Guide at pp-22

[13] 43 Fed Reg at 11112

"Birth Defects" listed.

[14] Guide at pp-22

NEUROTOXICITY	N	Y[15]
CARCINOGENICITY	Y[16]	Y[17]
MUTAGENICITY		
In Vitro	Y}[18]	Y} [19]
In Vivo	Y}	Y}
ENVIRONMENTAL		
Bioaccumulation	Y}	N
Bioconcentration	Y}[20]	N
Oct/water Part. Coeff.	Y}	N
Acute Fish	N	N
Acute Daphnia	N	N
Subchronic Fish	N	N
Subchronic Daphnia	N	N
Chronic Fish	N	N
AVIAN		
Acute	N	N
Reproductive	N	N
Reprodcutive	N	N

[15] Guide at pp-23; 33-34.
[16] 43 Fed Reg at 11112
"Cancer" listed
[17] Guide at pp-21.
[18] 43 Fed Reg at 11112; 11115 at Comment 15
"Mutagenicity" listed/ *in vivo* vs *invitro* discussed; discussion of "Ames test".
[19] Guide at pp-23.
[20] 43 Fed Reg at 11112; 11115 at Comment 16.

CAS #28064-14-4
Chem: Poly(formaldehyde/phenol)glycidyl ether
Title: Salmonella/Mammalian-Microsome Mutagenicity Test with TK 10 000
Date: May 31, 1979
Summary of Effects: Positive

CIBA-GEIGY Limited
Basle, Switzerland

SALMONELLA/MAMMALIAN-MICROSOME MUTAGENICITY TEST

with

TK 10 000

(Test for mutagenic properties in bacteria)

GU 2.3
May 31, 1979

Salmonella/Mammalian-
Microsome Mutagenicity Test

No. of experiment: 78/2606
Batch: Op.Nr.390974

SUMMARY AND CONCLUSIONS

TK 10 000 was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium. The investigations were performed with the following concentrations of the trial substance with and without microsomal activation: 25, 75, 225, 675 and 2025 µg/0.1 ml.

These tests permit the detection of point mutations in bacteria induced by chemical substances. Any mutagenic effects of the substances are demonstrable on comparison of the numbers of bacteria in the treated and control cultures that have undergone back-mutation to histidine-prototrophism. To ensure that mutagenic effects of metabolites of the test substances formed in mammals would also be detected, experiments were performed in which the cultures were additionally treated with an activation mixture (rat liver microsomes and co-factors)[1,2,3].

In the experiments performed without and with microsomal activation, the number of back-mutant colonies of Strains TA 100 and TA 1535 was significantly greater after treatment with TK 10 000 than in the controls.

Compound TK 10 000 thus exerted a mutagenic action in this test system.

P. Arni

(Dr. P. Arni)

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health
and Environment
Toxicology

(Prof.Dr.D.Müller)

Date: May 31, 1979

MATERIALS AND METHODS

The bacteria on which the tests were performed were the histidine-auxotrophic TA 98, TA 100, TA 1535 and TA 1537 strains of Salmonella typhimurium. Cultures were prepared from frozen stocks, and on the following days the Standard Plate Test was carried out with and without the addition of activation mixture (rat liver microsomes and co-factors)[1,2,3].

The test was performed with the following concentrations of the trial substance with and without microsomal activation: 25, 75, 225, 675 and 2025 µg/0.1 ml. The substance was dissolved in DMSO (Merck, Darmstadt, Germany, Art.No.2931). DMSO alone was used for the negative controls (the substances and vehicles used for the positive controls are indicated below). Each Petri dish contained: 1) approx. 20 ml of minimum agar (Agar purified, "Difco" certified, Difco Laboratories, Detroit, Michigan, U.S.A., Art.No.05650, plus salts (Vogel-Bonner Medium E) and glucose), 2) 0.1 ml of the solution of the test substance or the vehicle and 0.1 ml of a bacterial culture (in nutrient broth: Bacto Nutrient Broth dehydrated, Difco Laboratories, Detroit, Michigan, U.S.A., Art.No.0003 0.8% plus 0.5% NaCl) in 2.0 ml of soft agar. The soft agar was composed of: 100 ml of 0.6% agar solution (Agar purified, "Difco" certified) with 0.6% NaCl and 10 ml of a solution of l-histidine, 0.5 mM (Fluka, Buchs, Switzerland, Art.No.14400) and +biotin 0.5 mM (Fluka, Buchs, Switzerland, Art.No.53320). In the experiments in which the substance was metabolically activated, 0.5 ml of an activation mixture was added also[2,3]. 1 ml activation mixture contains: 0.3 ml S9 fraction of liver from rats induced with Aroclor 1254 (Analabs, Inc., North Haven, Connecticut, U.S.A., No.RCS-088), 8 µmoles $MgCl_2$, 33 µmoles KCl, 5 µmoles glucose-6-phosphate, 4 µmoles NADP and 100 µmoles phosphate buffer, pH 7.4.

Positive control experiments were carried out simultaneously with the following substances: 1) for Strain TA 98: daunoblastin (Soc.

Farmaceutici Italia, Milan, Italy), 5 and 10 µg/0.1 ml phosphate buffer; 2) for Strain TA 100: 4-nitroquinoline-N-oxide (Fluka, Buchs, Switzerland, Art.No.73265), 0.125 and 0.25 µg/0.1 ml phosphate buffer; 3) for Strain TA 1535: N-methyl-N'-nitro-N-nitrosoguanidine (Fluka, Buchs, Switzerland, Art.No.68051), 3 and 5 µg/0.1 ml phosphate buffer; 4) for Strain TA 1537: 9(5)aminoacridine hydrochloride monohydrate (Fluka, Buchs, Switzerland, Art.No.06650), 50 and 100 µg/0.1 ml DMSO. The activation mixture was tested with Strain TA 1535 and cyclophosphamide, 250 µg/0.1 ml phosphate buffer.

In the experiments with and without the addition of microsomal activation mixture three Petri dishes were prepared per strain and per group (i.e. per concentration or per control group).

The plates were incubated for about 48 hours at 37°C in darkness.

When the colonies had been counted, the arithmetic mean was calculated. The test substance was considered to be non-mutagenic if the colony count in relation to the negative control was not doubled at any concentration[3].

RESULTS

(see Tables 1 and 2)

In the experiments without microsomal activation, treatment with TK 10 000 led to an increase in the number of back-mutant colonies of Strains TA 100 and TA 1535. The highest number of back-mutant colonies was observed at the concentration of 2025 µg/0.1 ml.

In the experiments performed with microsomal activation on Strains TA 100 and TA 1535 treatment with TK 10 000 led to an increase in the number of back-mutant colonies. This effect was observed at the concentrations of 75 µg/0.1 ml and above in the experiment on Strain TA 1535 and at the concentrations of 675 and 2025 µg/0.1 ml in the experiment on Strain TA 100.

[1]AMES, B.N., F.D. LEE, and W.E. DURSTON (1973), An Improved Bacterial Test System for the Detection and Classification of Mutagens and Carcinogens.
Proc. Natl. Acad. Sci. USA 70, 782-786.

[2]AMES, B.N., W.E. DURSTON, E. YAMASAKI, and F.D. LEE (1973), Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection.
Proc. Natl. Acad. Sci. USA 70, 2281-2285.

[3]AMES, B.N., J. McCANN, and E. YAMASAKI (1975), Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test.
Mutation Res. 31, 347-364.

Table 1

Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments without microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

		Strain of S. typhimurium used			
		TA 98	TA 100	TA 1535	TA 1537
Test substance					
TK 10 000	Control	37	194	17	7
	25 µg/0.1 ml	47	178	20	8
	75 µg/0.1 ml	46	203	26	8
	225 µg/0.1 ml	39	221	49	7
	675 µg/0.1 ml	39	288	74	9
	2025 µg/0.1 ml	56	398	176	11
Positive controls					
Daunoblastin	Control	48			
	5.0 µg/0.1 ml	181			
	10.0 µg/0.1 ml	437			
4-Nitroquinoline-N-oxide	Control		204		
	0.125 µg/0.1 ml		665		
	0.25 µg/0.1 ml		∿1040		
N-Methyl-N'-nitro-N-nitroso-guanidine	Control			16	
	3 µg/0.1 ml			47	
	5 µg/0.1 ml			508	
9(5)Aminoacridine hydrochloride	Control				7
	50 µg/0.1 ml				54
	100 µg/0.1 ml				507

Table 2

Salmonella/Mammalian-Microsome Mutagenicity Test
Experiments with microsomal activation
Number (arithmetic mean) of colonies of
histidine-prototrophic back-mutants

		Strain of S. typhimurium used			
		TA 98	TA 100	TA 1535	TA 1537
Test substance					
TK 10 000	Control	53	145	14	16
	25 µg/0.1 ml	50	153	23	13
	75 µg/0.1 ml	37	186	47	10
	225 µg/0.1 ml	49	270	128	11
	675 µg/0.1 ml	52	488	337	12
	2025 µg/0.1 ml	62	724	652	15
Positive control of the microsomal activation					
Cyclophosphamide	Control			20	
	250 µg/0.1 ml			503	

Verteiler:

HH.	Dr. P. Arni	(2x)
	Prof.R.Hess	(1x)
	Dr. R. Leimgruber	(2x)
	Prof.D.Müller	(2x)

CIBA-GEIGY Limited
Basle, Switzerland

EPN 1139

POINT MUTATION ASSAY WITH MOUSE LYMPHOMA CELLS

with

TK 10 000

(In vitro test for mutagenic properties in mammalian cells)

EPA 1153

GU 2.3
February 7, 1980

Point mutation assay with
mouse lymphoma cells
(in vitro test)

No of experiment: 78-2331
Batch No: 390974

SUMMARY AND CONCLUSIONS

TK 10 000 was tested for mutagenic effects on mouse lymphoma cells (L5178Y) in vitro. The investigations were performed with the concentrations of 20.0 and 22.0 µg/ml.

This test system permits the detection of forward point mutations in mammalian cells induced by chemical substances. The gene changes are detected by three marker genes that are contained in the L5178Y-line of mouse lymphoma cells[1]. Mutagenic effects manifest themselves in the occurrence of mutants, expressed by resistance to any of three antimetabolites (methotrexate, cytosine arabinoside and thymidine). Any mutagenic effects of the substances are demonstrable on comparison of the number of clones in the treated and control cultures.

In the 4- and 18-hour toxicity test each a concentration producing an 80% cell-kill, best suited for mutagenicity testing, was used.

Comparison of the number of forward mutant colonies of the treated and control cells revealed differences attributable to treatment with the substance. These differences were present in the selection medium containing the antimetabolite thymidine.

Therefore, in this in vitro-test system, under the conditions described, TK 10 000 displayed a mutagenic activity.

Study director: (F.F. Strasser)
February 7, 1980

Report reviewed and approved by: (Prof.Dr. D. Müller)
(Head of Experimental Pathology)
Date: February 8, 1980

CIBA-GEIGY Limited
Basle, Switzerland
Protection of Health and Environment
Toxicology

PROCEDURE

A toxicity test was first performed in vitro to determine the concentrations to be used in the mutagenicity assay. The concentrations, best suited for mutagenicity test, are those causing an 80% cell-kill.

The L5178Y cells used in this assay were taken from cultures in an exponential phase of growth. The substance was prepared in DMSO solution and applied in Fischer's medium containing 10% horse serum in various doses ranging from 10 to 10 000 µg/ml, and the incubation was continued for 4 or 18 h in a 5 % CO_2 atmosphere in 5-ml Falcon flasks.

After the removal of the test substance, samples were taken for determination of viability. For each concentration, eight tubes each containing 100 cells in semisolid agar were prepared and incubated for 10 days at 37°C in a CO_2 atmosphere.

At the end of the incubation period a clone count was carried out and the number of clones in the control was set at 100 %. As a rule, from the results obtained, the concentrations required to produce an 80 % cell-kill is calculated.

For the 4 h incubation as well as for the 18 h incubation an 80 % cell-kill was obtained. Thus, in the mutagenicity experiments the concentrations of 22 µg/ml and of 20 µg/ml respectively were employed.

The mutagenicity test was carried out by treating L5178Y cells with the selected concentration at a cell density of 10^6/ml in Falcon flasks. The exposure time was 4 h and 18 h respectively. After removal of the substance, the cells were incubated for three days, the cell density being adjusted daily to $2x10^5$

cells/ml. At the end of the expression period, the cells were set up at a density of $4x10^5$ cells/5 ml in a semi-solid agar containing antimetabolites in culture tubes. Due to limited growth in the recovery phase, a cell density of $2x10^5$ cells/5 ml was seeded in the 18 h-experiment. The antimetabolites added were methotrexate (LEDERLE), thymidine (FLUKA) and cytosine arabinoside (SERVA).

Parallel with these cultures a cell-viability control was carried out. For this purpose 100 cells per 5 ml were seeded in agar of the same quality but without antimetabolites. The incubation time of the mutagenicity test cultures was 14 days, that for cell-viability control 10 days. The values obtained from the viability control are used to normalize the results received from the mutagenicity test, i.e. to preclude a 100%-viability of the cells seeded in cultures of the mutagenicity test.

The calculated mutant frequency corresponds to the number of clones per 100,000 cells. The mutation factor is calculated by dividing the mutant frequency of the treated cells by the mutant frequency of the control cells. The test substance is considered to be non-mutagenic if the mutation factor is not greater than 2.5.

RESULTS

(Table 1)

The cells were incubated together with the substance in a concentration of 22.0 µg/ml for four hours and of 20 µg/ml for 18 hours. These concentrations did produce the required 80 % cell-kill. The appropriate cell number of $4x10^5$ cells per test tube for the 18 hour mutagenicity study was not available, for slight cytotoxicity continued to appear during the three days of mutant expression. For this reason, only $2x10^5$ cells per test tube were disposable for cloning.

In the first set of 4 h experiments, the thymidine-variants were with a mutant factor reading of 6.11 transgressing the mutant factor treshold. In order to confirm the positive finding in this selective agent, three additional experiments using thymidine were performed. One of these experiments again proved a positive result with a mutant factor of 2.69.

[1]FISCHER, G.A., LEE, S.Y., and CALABRESI. P.: Detection of chemical mutagens using a host-mediated assay (L5178Y) mutagenesis system.
Mutation Research 26, 501-511 (1974).

able 1

OINT MUTATION ASSAY WITH MOUSE LYMPHOMA CELLS (IN VITRO TEST)

est substance	System	Dosage µg/ml	Antimetabolites					
			Cytosine-arabinoside		Methotrexate		Thymidine	
			MF	MFF	MF	MFF	MF	MFF
K 10 000	in vitro 4h	22.0	0.1		19.99		2.14	
		Control	0.06	1.67	11.06	1.81	0.35	6.11
	in vitro 4h	22.0					31.67	
		Control					16.14	1.96
	in vitro 4h	22.0					34.25	
		Control					12.71	2.69
	in vitro 4h	22.0					33.7	
		Control					16.43	2.05
	in vitro 18h	20.0	0.13		16.44		0.62	
		Control	0.39	0.33	11.49	1.43	0.44	1.41

1F = Mutant frequency per 100 000 cells.

1FF = Factor (MF dosage group/MF control)

Triage of 8(e) Submissions

Date sent to triage: 2/5/96

NON-CAP (CAP)

Submission number: 13141A

TSCA Inventory: (Y) N D

Study type (circle appropriate):

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ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

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TYPE: (INT.) SUPP FLWP

SUBMITTER NAME: E.I. Dupont de Nemours and Company

SUB. DATE: 09/11/92 OTS DATE: 09/22/92 CSRAD DATE: 03/24/95

CHEMICAL NAME:

CAS#
28064-14-4

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0678) CAP NOTICE

VOLUNTARY ACTIONS:
(0401) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP/USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
(0204)	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:

NON-CBI INVENTORY
(YES)
CAS SR
NO
IN TRAINING

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES

TOXICOLOGICAL CONCERN:
LOW
(MED)
HIGH

USE:

PRODUCTION:

COMMENTS

12) ✓ 8EHQ-92-13141: Rank - medium.

Chemical: poly(formaldehyde/phenol)glycidyl ether (CAS# 28064-14-4).

Salmonella/mammalian-microsome mutagenicity test with TK 10 000, Ciba-Geigy Ltd, Basle, Switzerland, dated May 31, 1979: Positive for gene mutations in Salmonella typhimurium in strains TA100 and TA1535 both without and with metabolic activation, negative with strains TA98 and TA1537 both without and with metabolic activation.